

***Callosus wenshanensis* gen. & sp. nov. from China**

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ABSTRACT—A new genus of corticoid wood-inhabiting fungus, *Callosus*, typified by *C. wenshanensis*, is proposed here based on morphological and molecular characters. The species is characterized by resupinate basidiomata with smooth, cream-colored hymenial surfaces, a monomitric hyphal system with generative hyphae bearing simple septa, and basidiospores ($2.3\text{--}3.9 \times 1.4\text{--}2.3 \mu\text{m}$) that are ellipsoid, hyaline, thin-walled, and smooth. The nrDNA internal transcribed spacer (ITS) and large subunit (28S) sequences were analyzed using maximum likelihood, maximum parsimony, and Bayesian inference methods. Phylogenetic analysis supported *Callosus* in a monophyletic lineage closely related to *Phanerochaete* and *Rhizochaete*.

KEY WORDS— *Phanerochaetaceae*, *Polyporales*, taxonomy, Yunnan Province

Introduction

Phanerochaetaceae Jülich (*Polyporales*, *Basidiomycota*) is a diverse family of fungi that grow on boreal, temperate, subtropical, and tropical vegetation (Dai 2012, Dai & al. 2015). The family is characterized by a monomitric hyphal system, generative hyphae without clamp-connections, and thin-walled smooth colorless basidiospores. Its species often possess cystidia and

cause white rot (Justo & al. 2017). Kirk & al. (2008) included 19 genera (+ 9 synonyms) in *Phanerochaetaceae*, but after phylogenetic analyses, Justo & al. (2017) cited 12 genera (+ one synonym), noting that “generic concepts in the family are in need of additional research.” Several molecular studies involving the *Phanerochaetaceae* have been conducted (Larsson 2007, Binder & al. 2013, Floudas & Hibbett 2015, Justo & al. 2017).

During a survey of wood-inhabiting fungi in southern China, some interesting collections were encountered that appeared to belong in *Phanerochaetaceae*. ITS and 28S sequence analyses supported these specimens as representing a new genus and species, proposed here as *Callosus wenshanensis*.

Materials & methods

The specimens included in the present study were deposited at the herbarium of Southwest Forestry University, Kunming, Yunnan Province, China (SWFC). Macromorphological descriptions were based on field notes. Color terms follow Petersen (1996). Micromorphological data were obtained from the dried specimens and observed under a Nikon light microscope. The following abbreviations are used: KOH = 5% aqueous potassium hydroxide (w/v), CB = Cotton Blue, CB- = acyanophilous, IKI = Melzer’s reagent, IKI- = both non-amyloid and non-dextrinoid, L = mean spore length (arithmetic average of all basidiospores), W = mean spore width (arithmetic average of all basidiospores), Q = variation in the L/W ratios, n = number of spores measured/number of specimens.

Genomic DNA was obtained from dried specimens using the CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd) according to the manufacturer’s instructions. ITS sequences were amplified using primers ITS5 and ITS4 (White & al. 1990) and 28S sequences using primers LR0R and LR7 (https://sites.duke.edu/vilgalyslab/rdna_primers_for_fungi/). The ITS was amplified as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles at 94 °C for 40 s, 58 °C for 45 s and 72 °C for 1 min, and a final extension of 72 °C for 10 min. The 28S region was amplified as follows: initial denaturation at 94 °C for 1 min followed by 35 cycles at 94 °C for 30 s, 48 °C for 1 min, and 72 °C for 1.5 min and a final extension of 72 °C for 10 min. The PCR amplicons were purified and directly sequenced by Kunming Tsingke Biological Technology Ltd. Co.

The resulting DNA sequences were deposited in GenBank (TABLE 1) and edited using Sequencher 4.6 (Gene Codes). The sequences were aligned using the “G-INS-i” strategy in MAFFT 7 (<http://mafft.cbrc.jp/alignment/server/>) and then manually adjusted in BioEdit (Hall 1999). The sequence alignment was deposited in TreeBase (Submission ID 27178). For phylogenetic analysis, sequences of *Candelabrochaete africana* Boidin were obtained from GenBank for use as outgroup (FIG. 1) following Floudas & Hibbett (2015). *Bjerkandera adusta* (Willd.) P. Karst. and *Ceriporiopsis carnegiae* (D.V. Baxter) Gilb. & Ryvar den were obtained for use as outgroup taxa (FIG. 2) following Justo & al. (2017).

TABLE 1. Species, specimens, and sequences used in this study. New sequences in **bold**.

SPECIES	VOUCHER	GENBANK ACCESSION NO.		REFERENCE
		ITS	nLSU	
<i>Bjerkandera adusta</i>	HHB 12826	KP134983	KP135198	Justo & al. 2017
<i>Callosus wenshanensis</i>	CLZhao 16017 T	MW553934	MW553936	This study
	CLZhao 16034	MW553935	MW553937	This study
<i>Candelabrochaete africana</i>	FP 102987	KP135294	KP135199	Floudas & Hibbett 2015
<i>Ceriporia purpurea</i>	KKN-223	KP135044	KP135203	Floudas & Hibbett 2015
<i>C. reticulata</i>	RLG-11354	KP135041	KP135204	Floudas & Hibbett 2015
<i>C. viridans</i>	Yuan 5702	KC182779	—	Floudas & Hibbett 2015
<i>Ceriporiopsis carnegieae</i>	RLG-7277	KY948792	KY948854	Justo & al. 2017
<i>Efibula americana</i>	FP-102165	KP135016	KP135256	Floudas & Hibbett 2015
<i>E. clarkii</i>	FD-228	KP135019	—	Floudas & Hibbett 2015
<i>E. gracilis</i>	FD-455	KP135027	—	Floudas & Hibbett 2015
<i>Hydnophlebia chrysorhiza</i>	FD-282	KP135338	KP135217	Floudas & Hibbett 2015
<i>H. omnivora</i>	KKN-112	KP135334	KP135216	Floudas & Hibbett 2015
<i>Phaeophlebiopsis caribbeana</i>	HHB-6990	KP135415	KP135243	Floudas & Hibbett 2015
<i>P. peniophoroides</i>	FP-150577	KP135417	KP135273	Floudas & Hibbett 2015
<i>Phanerochaete alnea</i>	Larsson 12054	KX538924	—	Floudas & Hibbett 2015
<i>P. arizonica</i>	RLG-10248	KP135170	KP135239	Justo & al. 2017
<i>P. australis</i>	HHB-7105	KP135081	KP135240	Floudas & Hibbett 2015

SPECIES	VOUCHER	GENBANK ACCESSION NO.		REFERENCE
		ITS	nLSU	
<i>P. chrysosporium</i>	HHB-6251	KP135094	KP135246	Justo & al. 2017
<i>P. ericina</i>	HHB-2288	KP135167	KP135247	Justo & al. 2017
<i>P. magnoliae</i>	HHB-9829	KP135089	KP135237	Justo & al. 2017
<i>P. pseudomagnoliae</i>	PP-25	KP135091	KP135250	Justo & al. 2017
<i>P. rhodella</i>	FD-18	KP135187	KP135258	Justo & al. 2017
<i>P. sordida</i>	FD-106	KP135070	KP135253	Floudas & Hibbett 2015
<i>P. subceracea</i>	FP-105974	KP135162	KP135255	Justo & al. 2017
<i>Phlebia acerina</i>	FD-301	KP135378	KP135260	Floudas & Hibbett 2015
<i>P. floridensis</i>	HHB-9905	KP135383	KP135264	Floudas & Hibbett 2015
<i>P. radiata</i>	AFTOL-484	AY854087	AF287885	Floudas & Hibbett 2015
<i>Phlebiopsis crassa</i>	KKN-86	KP135394	KP135215	Greslebin & al. 2004
<i>P. galochroa</i>	FP-102937	KP135391	KP135270	Justo & al. 2017
<i>P. gigantea</i>	FP-70857	KP135390	KP135272	Greslebin & al. 2004
<i>Rhizochaete brunnea</i>	MR-229	AY219389	AY219395	Greslebin & al. 2004
<i>R. filamentosa</i>	HHB-3169	KP135410	KP135278	Justo & al. 2017
<i>R. fouquieriae</i>	KKN-121	AY219390	AY219390	Floudas & Hibbett 2015
<i>R. radicata</i>	FD-123	KP135407	KP135279	Justo & al. 2017
<i>Scopuloides hydroides</i>	KHL-11916	EU118665	—	Floudas & Hibbett 2015
<i>S. rimosa</i>	RLG-5104	KP135351	KP135283	Floudas & Hibbett 2015

Maximum parsimony analysis was performed using PAUP* version 4.0b10 (Swofford 2002). All characters were equally weighted, and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Max-trees were set to 5000, branches of zero length were collapsed and all parsimonious trees were saved. Clade robustness was assessed using bootstrap (BT) analysis with 1000 replicates (Felsenstein 1985). Descriptive tree statistics, including tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each Maximum Parsimonious Tree (MPT) generated. Maximum Likelihood (ML) phylogenetic analysis was performed using RAxML-HPC2 via the Cipres Science Gateway (www.phylo.org; Miller & al. 2010).

For Bayesian inference (BI), the best-fit evolution model for each dataset was determined using MrModeltest 2.3 (Nylander 2004), and BI was performed in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003), using a general time reversible (GTR+I+G) model of DNA substitution and a gamma distribution for across-site rate variation. Four Markov chains were run for 2 runs from random starting trees for 900,000 for the ITS+28S dataset (FIG. 1) and for 50,000 for ITS+28S (FIG. 2) and trees were sampled every 100 generations. The first one-fourth generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. Branches were considered significantly supported if they received BS >70%, BT >50%, or BPP >0.95.

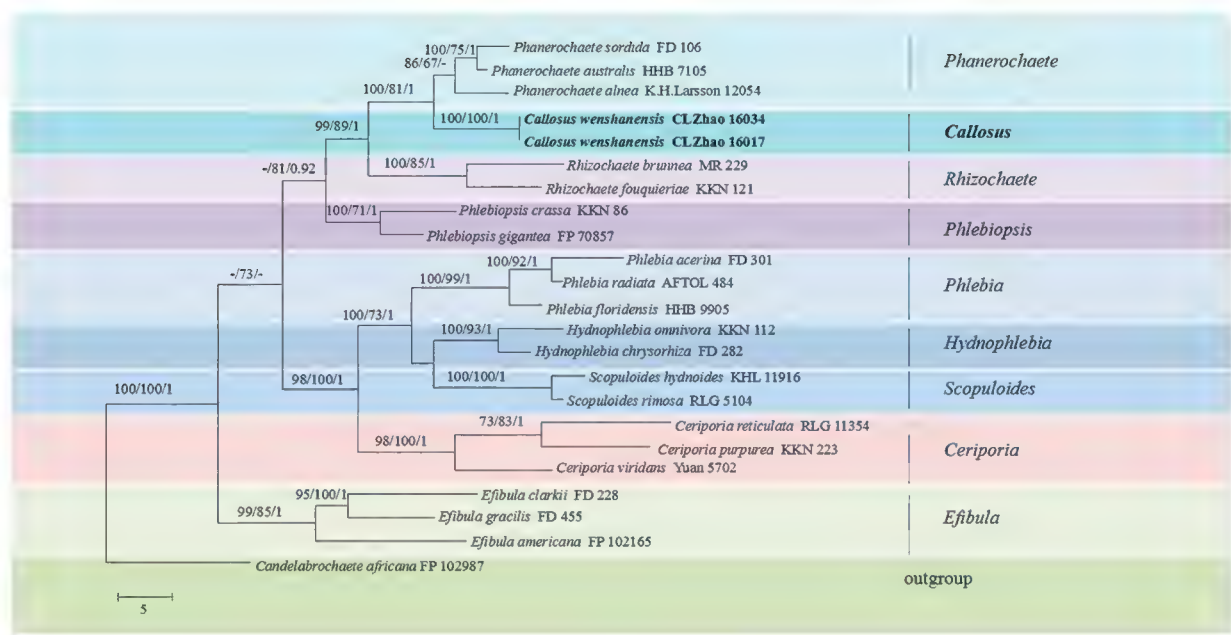


FIG. 1. Maximum parsimony strict consensus tree illustrating the phylogeny of *Callosus wenshanensis* and related genera in *Phanerochaetaceae* based on ITS+nLSU sequences. Generic names follow Floudas & Hibbett (2015). Branches are labelled with maximum likelihood bootstrap value >70%, parsimony bootstrap value >70% and Bayesian posterior probabilities >0.95.

Phylogenetic results

The 22 concatenated ITS+28S sequences used for phylogenetic analyses included sequences from representatives of *Efibula* Sheng H. Wu, *Ceriporia* Donk, *Hydnophlebia* Parmasto, *Phanerochaete* P. Karst., *Phlebiopsis* Jülich, *Phlebia* Fr., *Rhizochaete* Gresl. & al., and *Scopuloides* (Masse) Höhn. & Litsch. (FIG. 1)

The sequence alignment was 2260 bp long, of which 1627 were constant, 209 parsimony-uninformative, and 424 parsimony-informative. The MP analysis yielded two equally most-parsimonious trees (TL = 325, CI = 0.529, HI = 0.471, RI = 0.560, RC = 0.297). The best-fit BI model for the ITS+28S alignment was GTR+I+G [lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1)]. The BI and ML analyses yielded topologies similar to those yielded by MP analysis, with an average standard deviation of split frequencies equal to 0.009325.

Regardless of analysis method, the phylogenetic trees inferred from the ITS+28S sequences indicated that the specimens of interest formed a strongly supported monophyletic clade (BS = 100%, BT = 100%, BPP = 1) and were closely related to *Phanerochaete* and *Rhizochaete* (FIG. 1).

The ITS+28S dataset included sequences from 22 fungal specimens representing 21 taxa in *Phanerochaete*, *Phaeophlebiopsis* Floudas & Hibbett, *Phlebiopsis*, and *Rhizochaete* (FIG. 2).

The sequence alignment was 2130 bp long, of which 1680 were constant, 124 parsimony-uninformative, and 326 parsimony-informative. The MP analysis yielded two equally most-parsimonious trees (TL = 786, CI = 0.570, HI = 0.430, RI = 0.645, RC = 0.367). The best-fit BI model for ITS+28S alignment was GTR+I+G [lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1)]. The BI and ML analyses yielded topologies that were similar to those yielded by MP analysis, with an average standard deviation of split frequencies equal to 0.008889.

Regardless of analysis method, the phylogenetic trees inferred from the ITS+28S sequences placed the specimens of interest in a strongly supported monophyletic clade (BS = 100%, BT = 100%, BPP = 1) closely related to *Phanerochaete* and *Phaeophlebiopsis* (FIG. 2).

In addition, BLAST results comparing ITS sequences from the Chinese specimens and species in the NCBI database showed a high similarity with *Phanerochaete* sp. (Maximum & total score 749; Query coverage 93%; E value 0; Identity 88.58%) and *P. sordida* (P. Karst.) J. Erikss. & Ryvarden (Maximum & total score 734; Query coverage 87%; E value 0; Identity 89.24%). Those

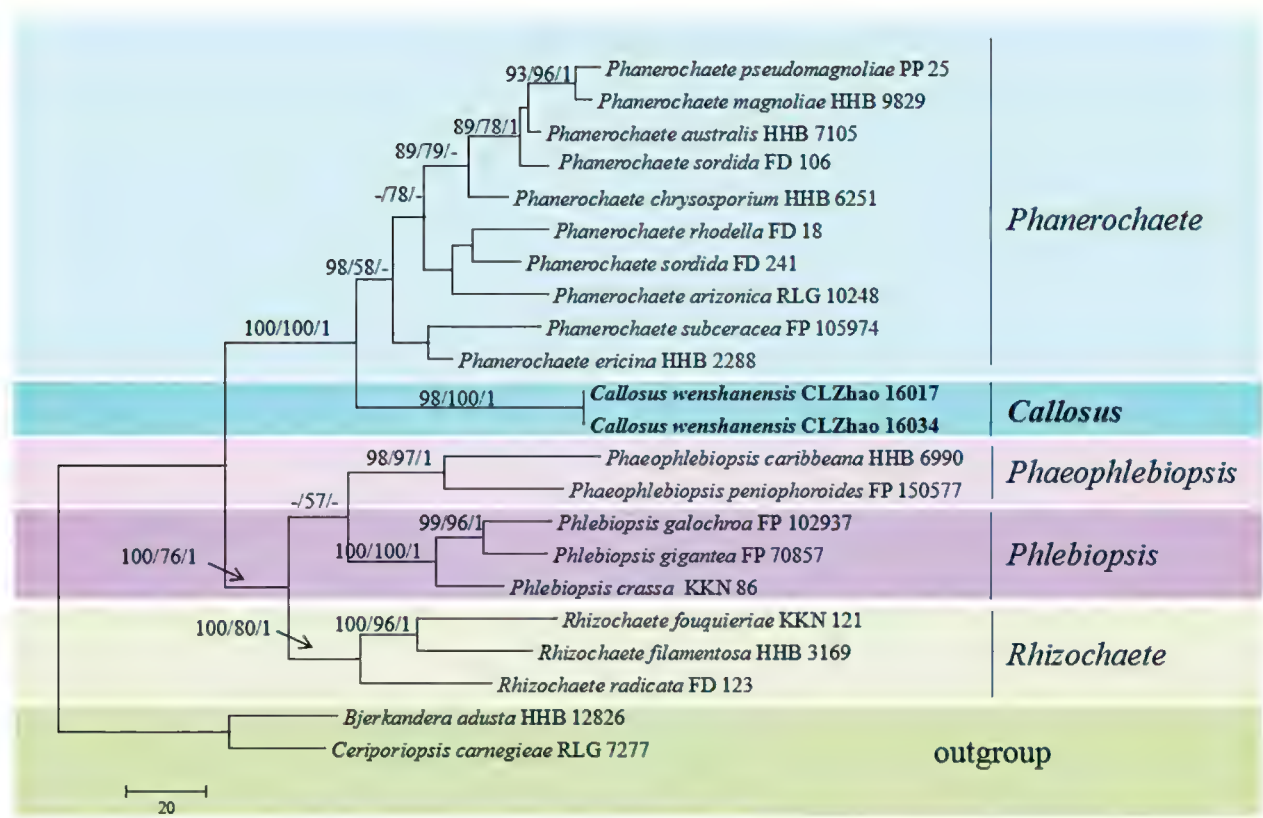


FIG. 2. Maximum parsimony strict consensus tree illustrating the phylogeny of *Callosus wenshanensis* and related species in *Phanerochaetaceae* based on ITS+nLSU sequences. Generic names follow Justo & al. (2017). Branches are labelled with maximum likelihood bootstrap value >70%, parsimony bootstrap value >70% and Bayesian posterior probabilities >0.95.

comparing 28S sequences showed a high similarity with *Phanerochaete* sp. (Maximum & total score: 2412; Query coverage 99%; E value 0; Identity 98.19%) and *P. cystidiata* Sheng H. Wu & al. (Maximum & total scores 2409; Query coverage 98%; E value 0; Identity 98.47%).

Taxonomy

Callosus C.L. Zhao, gen. nov.

MB 838558

Differs from *Phanerochaete* by its membranous basidiomata with a lubricative hymenial surface and the presence of crystals between subhymenium and subiculum.

TYPE SPECIES: *Callosus wenshanensis* C.L. Zhao

ETYMOLOGY: *Callosus* (Lat.) refers to the hard membranous basidioma and its smooth hymenophore.

BASIDIOMATA annual, resupinate, membranous. Hymenial surface smooth, white to cream. Hyphal system monomitic; generative hyphae bearing simple septa, IKI–, CB–; tissues unchanged in KOH. Cystidia capitate, cystidioles

absent. Basidia clavate to cylindrical, with four sterigmata. Basidiospores ellipsoid, colorless, thin-walled, smooth, IKI–, CB–.

TYPE OF ROT: white rot.

***Callosus wenshanensis* C.L. Zhao, sp. nov.**

FIGS 2, 3

MB 838559

Differs from *Phanerochaete alnea* by its white to cream-colored basidiomata and smaller basidiospores.

TYPE: China. Yunnan Province, Wenshan, Baxin Town, Wenshan National Nature Reserve, on fallen angiosperm branch, 24 July 2019, C.L. Zhao 16017 (Holotype, SWFC 0016017; GenBank MW553934, MW553936).

ETYMOLOGY: *wenshanensis* (Lat.) refers to the provenance of the specimens.

BASIDIOMATA annual, resupinate, membranous, without odor or taste when fresh, becoming hard membranous and fragile upon drying, ≤10 cm long, 2.5 cm wide, 1 mm thick. Hymenial surface smooth, white to cream-colored when fresh, becoming cream-colored and cracking upon drying. Margin sterile, narrow, 1 mm wide, white to cream.

HYPHAL STRUCTURE monomitic; generative hyphae with simple septa, colorless, thin- to thick-walled, unbranched, 1.5–2.3 µm in diameter, IKI–, CB–; tissues unchanged in KOH.

SUBICULUM subhymenium and subiculum distinct, subicular hyphae loosely arranged, subhymenium covered with crystals.

HYMENIUM cystidia capitate, colorless, thin-walled, progressively broader towards the top, 23.5–27.2 × 3.2–3.7 µm. Basidia clavate to cylindrical, with four sterigmata, 16.2–17.1 × 2.7–3.4 µm, basidioles dominant, similar in shape to basidia, but slightly smaller.

BASIDIOSPORES ellipsoid, colorless, thin-walled, smooth, with 1–2 bubbles, IKI–, CB–, 2.3–3.9 (–4.1) × 1.4–2.3 µm, L = 3.02 µm, W = 1.73 µm, Q = 1.68–1.82 (n = 60/2).

TYPE OF ROT: white rot

ADDITIONAL SPECIMEN EXAMINED: CHINA. YUNNAN PROVINCE. Wenshan: Baxin town, Wenshan National Nature Reserve, on fallen angiosperm branch, 24 July 2019, C.L. Zhao 16034 (SWFC 0016034; GenBank MW553935, MW553937).

Discussion

The proposed new genus, *Callosus*, is supported based on both phylogenetic analyses and morphological characters.

Floudas & Hibbett (2015) revised the taxonomy of *Phanerochaete* (*Polyporales*, *Basidiomycota*) using four nuclear ribosomal gene markers (RPB1,

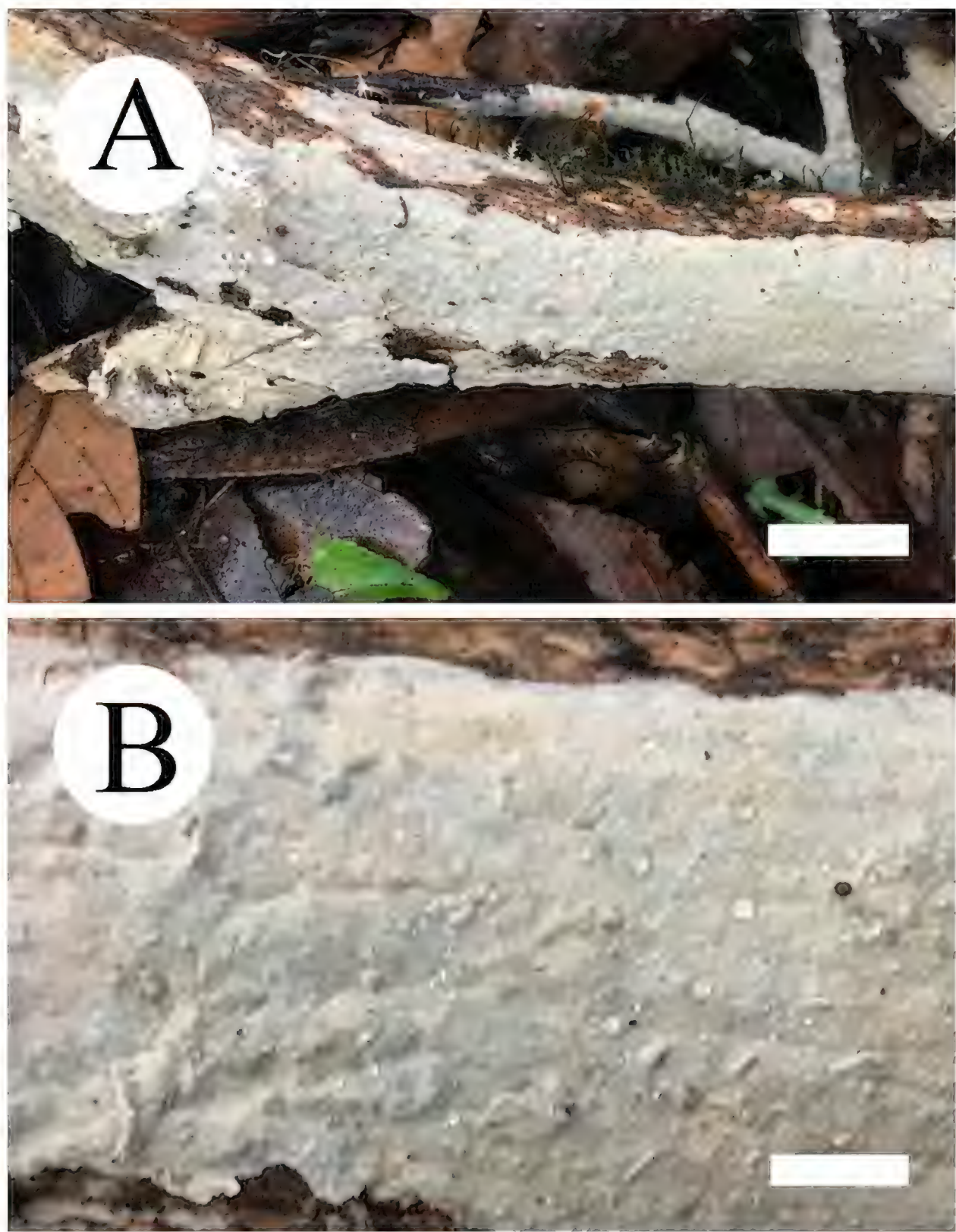


FIG. 3. *Callosus wenshanensis* (holotype, SWFC 0016017).
A. Habit; B. Characteristic hymenophore. Scale bars: A = 1 cm; B = 0.5 cm.

RPB2, ITS and 28S), in which *Hydnophlebia*, *Phaeophlebiopsis*, *Phanerochaete*, *Phlebia*, *Phlebiopsis*, *Rhizochaete*, and *Scopuloides* grouped together within the residual polyporoid clade. The present study found that ITS+28S sequences

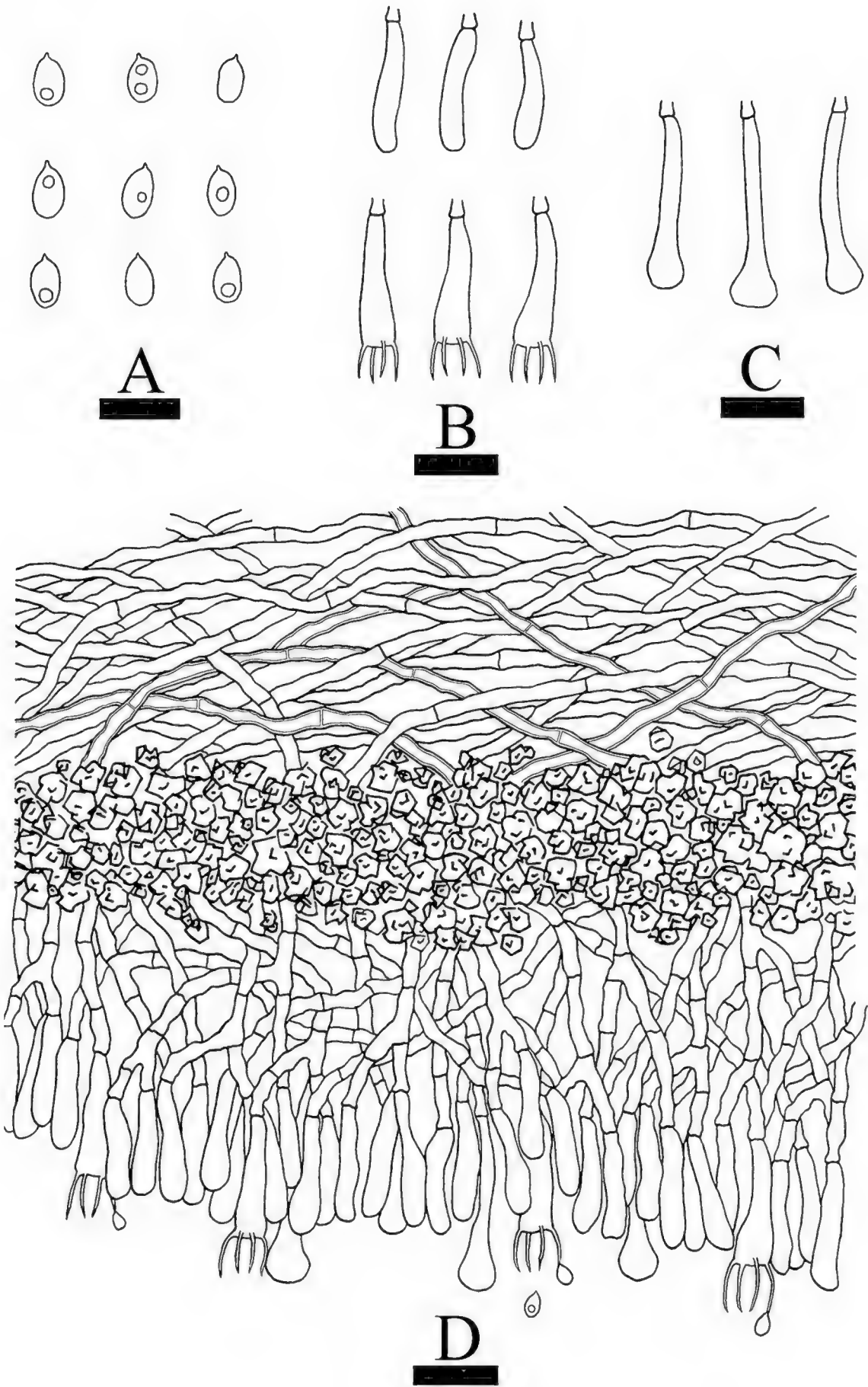


FIG. 4. *Callosus wenshanensis* (holotype, SWFC 0016017).
A. Basidiospores; B. Basidia and basidioles; C. Cystidia; D. Section of hymenium.
Scale bars: A = 5 μ m, B–D = 10 μ m.

from *Callosus wenshanensis* form a strongly supported monophyletic lineage closely related to *Phanerochaete* and *Rhizochaete* (FIGS 1 & 2). However, *C. wenshanensis* differs morphologically from *P. alnea* (Fr.) P. Karst., which is distinguished by its pale pink to pale ochraceous basidiomata, basidioma with fibrillose margin, short hyphal strands, and larger basidiospores ($4.9\text{--}7.5 \times 2.9\text{--}4 \mu\text{m}$, Spirin & al. 2017), and from *Rhizochaete brunnea* Gresl. & al., distinguished by tuberculate hymenial surfaces, tissues that turn violet in KOH, brown cystidia, clamped generative hyphae, and larger basidiospores ($5\text{--}6.5 \times 3\text{--}3.5 \mu\text{m}$, Greslebin & al. 2004). A morphological comparison between *Callosus* and related genera is presented in TABLE 2.

Corticoid fungi are extensively studied and well-known worldwide (Bernicchia & Gorjón 2010; Dai 2012; Dai & al. 2015), but Chinese corticoid fungi diversity is still being explored, especially in subtropical and tropical areas. *Callosus wenshanensis* is from Yunnan Province, where many new taxa in *Phanerochaetaceae* have been described (Wu & al 2010; Zhao & Ma 2019; Ma & Zhao 2019 Ma & al. 2020; Xu & al. 2020). We anticipate that additional, undescribed corticoid fungi will be discovered throughout China after extensive collection combined with morphological and molecular analyses.

Acknowledgements

Special thanks are due to Drs. Shah Hussain (Center for Plant Sciences and Biodiversity, University of Swat, Pakistan) and Lu-Sen Bian (Experiment Center of Forestry in North China, Chinese Academy of Forestry, P.R. China) for expert presubmission review. The research was supported by the National Natural Science Foundation of China (Project No. 32170004), Yunnan Fundamental Research Project (Grant No. 202001AS070043), the High-level Talents Program of Yunnan Province (YNQRQNRC-2018-111).

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TABLE 2. A morphological comparison of *Callosus* with closely related genera.

GENUS	FRUITBODY	HYMENOPHORE	CLAMP CONNECTIONS	HYPHAL SYSTEM	CYSTIDIA	SUBICULUM	REFERENCE
<i>Callosus</i>	Resupinate, adnate, and hard	Smooth	Absent	Monomitic	Capitate	Loosely arranged, covered with crystals	This study
<i>Efibula</i>	Resupinate	Smooth	Absent	Monomitic	Absent	Compact	Wu 1990
<i>Hydnophlebia</i>	Resupinate	Odontoid	Rare	Monomitic	Leptocystidia	Well-developed, loose	Parmasto 1967
<i>Phaeophlebiopsis</i>	Resupinate, and hard	Smooth	Rare or absent	Monomitic	Subulate or capitate	Well-developed, loose	Floudas & Hibbett 2015
<i>Phanerochaete</i>	Resupinate to slightly reflexed	Smooth, tuberculate or odontioid	Rare or absent	Monomitic	Present	Well-developed, loose	Karsten 1889
<i>Phlebiopsis</i>	Resupinate	Smooth to tuberculate	Absent	Monomitic	Metuloids	Compact	Jülich 1978
<i>Rhizochaete</i>	Resupinate	Smooth or slightly tuberculate	Absent, occasional, or present	Monomitic	Mostly present	Well-developed, loose	Greslebin & al. 2004
<i>Scopuloides</i>	Resupinate	Odontoid	Absent	Monomitic	Metuloids	Almost absent, compact	Burdsall 1985

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